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(54) Title: SOLUTION CONTAINING IGF-I

(57) Abstract

The invention relates to a stable solution containing Insulin-like Growth Factor I (IGF-I) or any functional analogue thereof as active agent and mannitol in a pH adjusted solution, optionally with a preservative. There should be no sodium chloride. This solution is physically stable without giving any precipitates, it is isotonic and suitable for injection.

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# SOLUTION CONTAINING IGF-I

The present invention relates to a stable solution containing Insulin-like Growth factor I (IGF-1) or any functional analogue thereof as active agent and mannitol in a pH adjusted solution, optionally with a preservative. There should be no sodium chloride. This solution is physically stable without giving any precipitates, it is isotonic and suitable for injection.

## Introduction

Insulin-like Growth Factor I (IGF-I) is a peptide present in plasma and other body fluids as well as many cells/tissues. It comprises 70 amino acids, including 3 disulphide bonds, and can stimulate proliferation of a wide range of cell types and it mediates some of the effects of growth hormone. Human IGF-I has been purified from plasma and its complete amino acid sequence is established. (Rinderknecht E et al. "The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin" J. Biol. Chem 253; 2769-76, 1978) Sequences with extensive homologies to human IGF-I are present in IGF-I purified from plasma of other species.

Because of the scarcity of purified plasma IGF-I there was a great necessity to develop methodology for the commercial scale production of IGF-I. Nowadays, such large scale production can readily be achieved by using recombinant DNA techniques.

As a result of studies with preparations of recombinant DNA IGF-I it has been demonstrated that it promotes skeletal growth and skeletal muscle protein synthesis. IGF-I has been shown to act both as an endocrine factor as well as a paracrine/autocrine factor. (Skottner et al, Endocrinology, Vol. 124, No 5, 1989 and Cook et al, J Clin Invest 81; 206-212; 1988)

Moreover, IGF-I is also effective for the treatment or prevention of catabolic states in patients (Swedish patent application SE 9002731-9) and improves the regeneration of transected peripheral nerves (EP 0 308 386).
It has previously been demonstrated *in vitro* that IGF-I also can promote actin synthesis in myocytes in culture (Florini, J R, Muscle and Nerve 10 (1987) 577-598 and contractility of neonatal rat cardiocytes *in vitro* (Vetter, U *et al.*, Basic Res. Cardiol. 83 (1988)647-654).

The stability of proteins is generally a problem in the pharmaceutical industry. A formulation with a low amount of protein will generally lose activity during purification, sterile manufacturing, storage and during the administration.
It has often been solved by drying of the protein in different drying processes, such as freeze-drying. The protein has thereafter been distributed and stored in dried form. The patient necessarily has to reconstitute the dried protein in a solvent before use, which of course is a disadvantage and is an inconvenience for the patient.
For a patient, who needs daily injections of IGF-I, and especially when the patient is a child, it is of importance that the product is easy to handle, to dose and inject. The reconstitution of a freeze-dried product demands prudence and carefulness and should therefore preferably be avoided.
The freeze-drying process is also a costly and time consuming process step, and it would be of great advantage if this step could be avoided, when preparing a commercial product of a protein.

It would thus facilitate the use of a pharmaceutical protein if it can be produced and distributed as a stable solution with a prolonged storage life to the patient, who could inject the medicament directly without reconstitution.
It would be advantageous if the final pharmaceutical solution only contained a minimum of additives.

Proteins are different with regard to physiological properties. When preparing a pharmaceutical preparation which should be physiologically acceptable and stable for a long time, consideration can not only be taken to the physiological properties of the protein but also other aspects must be considered such as the industrial manufacture, easy handling for the patient and safety for the patient. The results of these aspects are not predictable when testing different formulations and each protein has often a unique solution regarding stability.

Mannitol has been mentioned as stabilizing agent in different peptide compositions. See e.g. EP 35 204 B ( Miles), EP 308 238 ( Ethicon), WO 9321908 ( Amgen) and EP 523 330 (American Cyanamid). The use of mannitol as stabilizing agent is to protect the protein from deamidation.

In WO 89/09614 (Genentech), a stabilized formulation of human growth hormone containing glycine, mannitol, optionally a non-ionic surfactant and a buffer at pH 4-8 is disclosed. The non-ionic surfactant is added for reduced aggregation and denaturation. The formulation has an increased stability in lyophilized form and as a solution obtained after reconstitution.

WO 9118621 (Genentech) discloses GH in a solution containing mannitol and phosphate buffer.

Mannitol as pharmaceutical agent is given to patients to decrease excess water (oedema) especially when intracranial hypertension is present. The mechanism is suggested to be mainly due to changes in peripheral osmolarity. Mannitol has been shown to pass the blood-brain barrier to some extent in normal individuals, but to larger extent in individuals with brain oedema. Transport of mannitol into the brain was shown to accelerate due to increased vesicular transport via the blood-brain barrier (Watanabe A, Res Exp Med (1992), 192; 401-406.)

Mannitol has been used in combination with nimodipine (a calcium antagonist) in experimental brain surgery and it was shown that the combined treatment was superior to either product alone as well as to controls in preserving cerebral blood flow and evoked potentials (Andrews RJ et al. Neurological Res (1992), 14; 19-25).

Different compositions containing IGF-I are known.

EP 440 989 (FUJISAWA) discloses a method for preparing a dried composition of IGF-I, which comprises drying a solution containing IGF-I together with a strong acid.

IGF-I in a citrate buffer at pH 6 is known from WO 91/18621, Genentech. Nothing is mentioned regarding stability of IGF-I.

WO 94/15584 (Pharmacia) discloses stable solution containing IGF-I. The use of a phosphate buffer in an amount of 50 mmol/L giving a pH of 5.5 to 6.5 gave advantages over solutions with other pH. It is stated that benzyl alcohol did not affect stability.

In one example (example 3) glycine was added to a water solution of 0.75 mg IGF/L with or without NaCl at pH 9. The results indicated that the presence of sodium chloride has a slightly positive effect in the stability of IGF-I in the buffered solution.

In example 4 a comparison is made with a composition containing 1 mg IGF-I /ml, phosphate buffer and NaCl or glycine at pH 7. It is stated that the addition of glycerol instead of NaCl decreased the stability of IGF-I.

These patents/applications do not to disclose any specific information related to the unique combination of IGF-I and mannitol without sodium chloride.

We have now found that IGF-I together with NaCl and phosphate can give a denaturation of the protein and structural three dimensional changes with subsequent aggregation in the protein. This can be seen on Differential Scanning Calorimetry (DSC).
In a solution containing NaCl and IGF-I in higher concentrations, i.e. above 5 mg/L, a precipitation can be seen

We have also found that IGF-I and mannitol gives a stable solution which does not form aggregates or is oxidated.

We have thus to our great surprise found that the addition of mannitol gives an unexpected positive effect for stability when compared to other stabilising agents such as glycerol and glycine and that it is preferable not to add NaCl .
No physical degradation occurs when mannitol is added, but NaCl gives physical degradation.
This is a surprising finding, which could not have been foreseen by a person skilled in the art, although mannitol earlier has been suggested as additive to peptide solutions.
The addition of benzylalcohol to our composition is based on the necessity from Health Authorizations to include a preservative.

For solutions intended for subcutaneous injection, pain can be a problem, especially if the pH of the solution deviates from the physiological pH. For stability reason of the active substance it can still be necessary to choose a pH deviating from the physiological pH. For such solution, a mean to overcome the pain upon injection would be most important, especially of the drug is to be injected regularly for many years, e.g. IGF-I.
pH and osmolarity is of importance for solutions to be injected without pain.

Underlying this invention is also to find a stable solution for IGF-I which does not hurt when injected and in stable in both physical and chemical respects.

The following figures are annexed:

Figure 1 DSC for two different solutions according to Examples 1 and comparative Example 2.

Figure 2. Light blocking particles in hIGF-I solution according to Example 3.

**The invention**

The invention relates to a stable solution comprising IGF-I or any functional analogue thereof as active agent and mannitol but without sodium chloride in a pH adjusted solution, optionally with a preservative.

The claimed solution has at least 95% of its original concentration value after storage for 2 months at +25±1°C and more preferably for 6 months +25±1°C.

Preferably rIGF-I is the active agent and more preferably the concentration of IGF-I is of at least 2 mg/mL.
Benzyl alcohol could be used as preservative.

The invention also relates to a process for preparation of the formulation by mixing IGF-I or any functional analogue thereof with mannitol, a buffer and optionally a preservative. It also relates to a method for treatment of a patient in need of IGF-I or any functional analogue thereof by administering the claimed formulation.

By Insulin-like Growth Factor (IGF-I) is meant both naturally occurring human and animal IGF-I and recombinant IGF-I (rIGF-I), such as rhIGF-I (human), rbIGF-I

(bovine) and rpIGF-I (porcine). By functional analogues are meant compounds having the same therapeutic and biological action as as IGF-I in animals and humans and having at least 65 % homology with natural occuring IGF-I.

The concentration of IGF-I is only dependent of its solubility in the used buffer and the desired therapeutically amount for the given dose. Preferably the concentration of IGF-I is 2-100 mg/mL and more preferably 5-20 mg/mL.

## Examples

The recombinant human IGF-I (rhIGF-I) used in the experiments was produced by Pharmacia. rhIGF-I can e.g. be produced in yeast or E Coli. Correctly processed and secreted rhIGF-I could be isolated from the fermentation media in its native form.

The media with rhIGF-I was micro filtered and impurities were removed by several chromatographic techniques known within the field.

In the following examples, solutions of IGF-I pools from the final step in the purification process were ultrafiltered to obtain a correct concentration and the correct buffer formulation.

The samples were stored at +7±1°C, 25±1°C or 37±3°C.

The following analytical techniques were used in all examples:

*Reversed Phase HPLC* (RP-HPLC) The elution system is composed of acetonitrile, water, phosphate buffer and propane sulphonic acid sodium salt. Elution is accomplished by decreasing the polarity of the mobile phase. UV

detection at 220 nm. Used for measurement of concentration and purity of IGF-I. The remains of the original concentration is calculated in per cent.
If the remains of the original concentration (calculated in per cent) follows the decrease of purity, only a chemical degradation has occurred. If the remains of the original concentration is decreased more rapid that purity, also a physical degradation has occurred.

*Differential Scanning Calorimetry* (DSC). The thermodynamic property which is determined on a DSC-instrument is the heat capacity, $C_p$ ($C_p = dH/dT$; $H$=Enthalpy and $T$=Temperature). For a protein solution this property can give qualitative and quantitative information about physiochemical processes, like unfolding and aggregation. Temperatures scans were run between +5°C and +75°C at a rate of 43°C/hour. Reference solutions were the corresponding buffers.

*pH* was carried out as prescribed in Ph. Eur. 2nd Ed.

Example 1.

This example presents the results from a stability study of a solution according to the invention which has been stored at +7, +25 and +37°C.

Composition per mL:

| | |
|---|---|
| IGF-I | 10 mg |
| mannitol | 15.6 mg |
| Benzyl alcohol | 12 mg |
| Phosphate buffer | 10 mmol/L |
| Water for injection | to make 1.0 mL |
| pH | 5.9 |

1 mL of this solution was filled in sterile glass vials.

All samples were stored protected from light and investigated.

RESULTS. The results after storage at +7, +25 and +37°C are presented in tables 1a, 1b and 1c respectively.

Table 1a. 10 mg/mL IGF-I stored at +7°C

| TIME months | RP-HPLC mg/mL | RP-HPLC % of orig. conc. |
|---|---|---|
| 0 | 10.3 | |
| 3 | 10.3 | 100 |
| 6 | 10.3 | 100 |
| 24 | 10.3 | 100 |

Table 1b 10 mg/mL IGF-I stored at +25°C

| TIME months | RP-HPLC mg/mL | RP-HPLC % of orig. conc. |
|---|---|---|
| 0 | 10.3 | |
| 1 | 10.5 | 100 |
| 2 | 10.2 | 99 |
| 6 | 10.2 | 99 |

Table 1c 10 mg/mL IGF-I stored at +37°C

| TIME months | RP-HPLC mg/mL | RP-HPLC % of orig. conc. |
|---|---|---|
| 0 | 10.3 | |
| 0.5 | 9.8 | 95 |
| 1 | 9.4 | 95 |

At +37°C 95 % of IGF-I concentration remained after 1 month. No aggregates or precipitation. The purity of the IGF-I decreased due to degradation reactions.

CONCLUSION

This study shows that a solution according to the invention is physically stable at +7 °C for at least 6 months.

The study also shows that a solution according to the invention is physically stable at 25 °C for at least 2 months.

Here the remains of the original concentration follows the decrease of purity, and thus only a minor chemical degradation has occurred.

Example 2, Comparative

The purpose of this study was to compare the stability of IGF-I formulated in an aqueous solution with benzyl alcohol and NaCl as comparison.

Composition 2:

| | |
|---|---|
| IGF-I | 10 mg/mL |
| Phosphate buffer | 10 mmol/L |
| NaCl | 8.48 mg/mL |
| benzylalcohol | 12 mg/mL |
| Water for injection | to make 1.0 mL |
| pH | 5.9 |

RESULTS. The results after storage at +7°, +25 and +37°C are presented in tables 2a, 2b and 2c respectively.

Table 2a 10 mg/mL IGF-I stored at +7°C

| TIME months | RP-HPLC mg/mL | RP-HPLC % of orig. conc. |
|---|---|---|
| 0 | 10.1 | |
| 3 | 8.5 | 84 |

Table 2b 10 mg/mL IGF-I stored at +25°C

| TIME months | RP-HPLC mg/mL | RP-HPLC % of orig. conc. |
|---|---|---|
| 0 | 10.1 | |
| 1 | 8.3 | 82 |
| 2 | 8.2 | 81 |

Table 2c 10 mg/mL IGF-I stored at +37°C

| TIME months | RP-HPLC mg/mL | RP-HPLC % of orig. conc. |
|---|---|---|
| 0 | 10.1 | |
| 0.5 | 8.5 | 84 |
| 1 | 8.0 | 79 |

CONCLUSION

This composition is not physically stable. Some visual precipitation and aggregates are formed and the IGF-I concentration decreases.

The solutions described in example 1 and comparative example 2 were analysed by DSC. The heat scans are shown in Figure 1.

The protein tends to aggregate resulting in an increasing heat capacity for the solution in comparative example 2, starting at 20-30°C. The solution in exemple 1 does not aggregate, which is shown by the low heat capacity in the temperature range studied.

This shows that the mannitol formulation, without NaCl is very stable towards precipitation and aggregation.

When tables 1a-c 1 are compared with tables 2a-c it is clearly seen that the addition of mannitol gives an effect on the physical stability, which cannot be seen when no mannitol but NaCl is present in the solution.

In comparative example 2 the remains of the original concentration is decreased more rapid that purity, thus also a physical degradation has occurred.

Example 3

Human IGF-I was mixed with excipients as described in the table below to make aqueous solutions of hIGF-I 10 mg/mL.

| Solution | Excipient | Concentration |
|---|---|---|
| A | Sodium chloride | 145 mM |
| B | Mannitol | 290 mM |
| C | Sodium chloride | 145 mM |
| | + benzyl alcohol | 140 mM |
| D | Mannitol | 290 mM |
| | + benzyl alcohol | 140 mM |

The solutions were allowed to equilibrate at 7°C for 48 hours and were then heated in aliquots of 5 mL at 25°C or 75°C for 1 hour. Immediately after the heating the particle content in the solutions was determined by Light Obscuration technique (HIAC/ROYCO 3004A equipped with a liquid sensor HR-LD 150 and a 9064 sizing counter, USA).

RESULTS

The counts of particles larger than 1.24um per mL are presented in figure 2. The solutions with mannitol exhibited a low particle content compared to the solutions with sodium chloride.

CONCLUSION

Mannitol stabilizes hIGF-I in aqueous solutions.

# CLAIMS

1. Stable solution comprising Insulin-like Growth Factor I (IGF-I )or any functional analogue thereof as active agent together with mannitol but without Sodium chloride in a pH adjusted solution, optionally with a preservative.

2. Stable solution according to claim 1 having at least 95% of its original concentration value after storage for 2 months at +25±1°C and more preferably for 6 months +25±1°C.

3. Stable solution according to any of claims 1 to 2 in which rIGF-I is the active agent.

4. Stable solution according to any of claims 1 to 3 in which the concentration of IGF-I is at least 2 mg/mL.

5. Stable solution according to any of claims 1 to 4 which contains benzyl alcohol as preservative.

6. A process for preparation of the stable solution according to any of claims 1 - 5 by mixing IGF-I or any functional analogue thereof with mannitol and optionally a preservative in water.

7. A method for treatment of a patient in need of IGF-I or any functional analogue thereof by administering the stable solution according to any of claims 1-5.

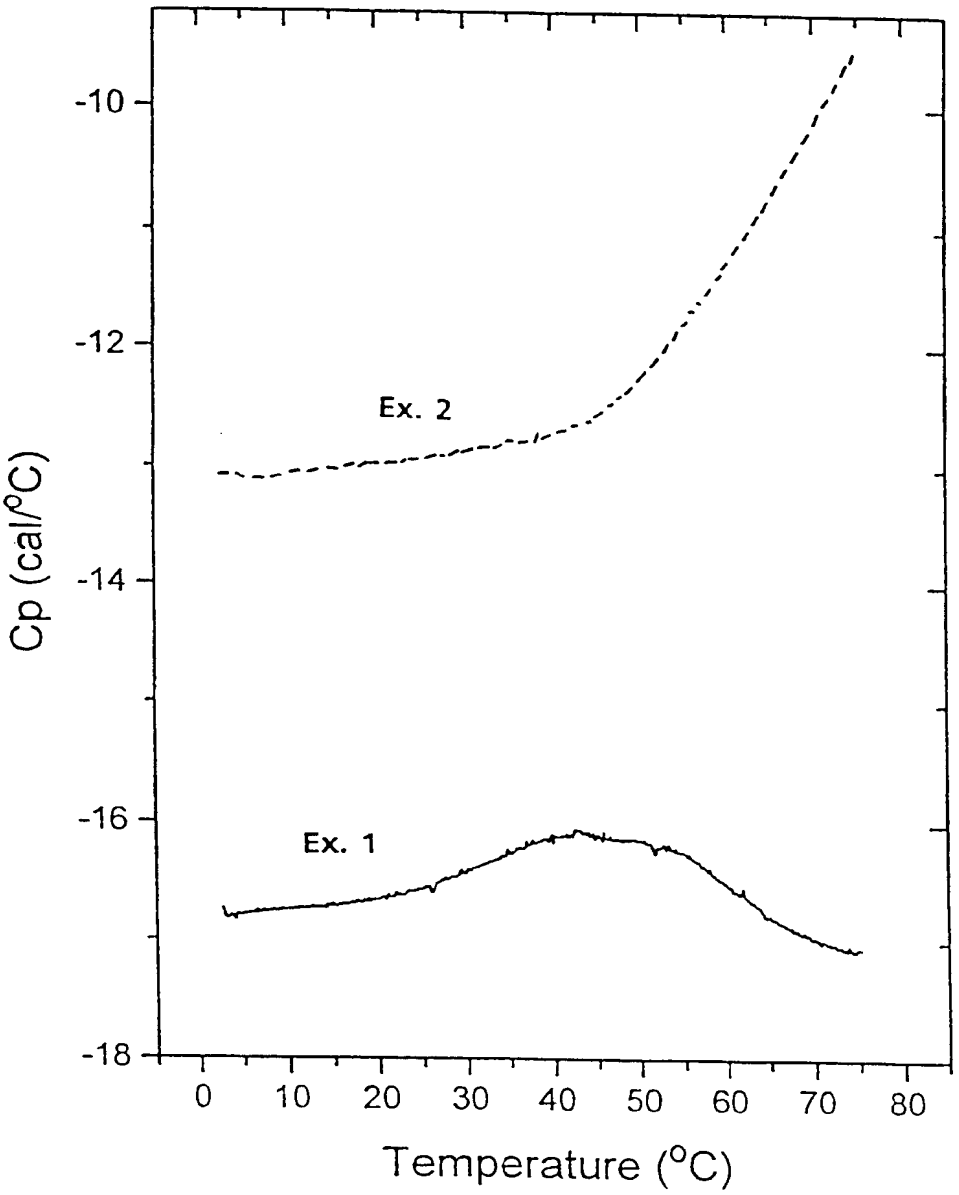


Figure 1

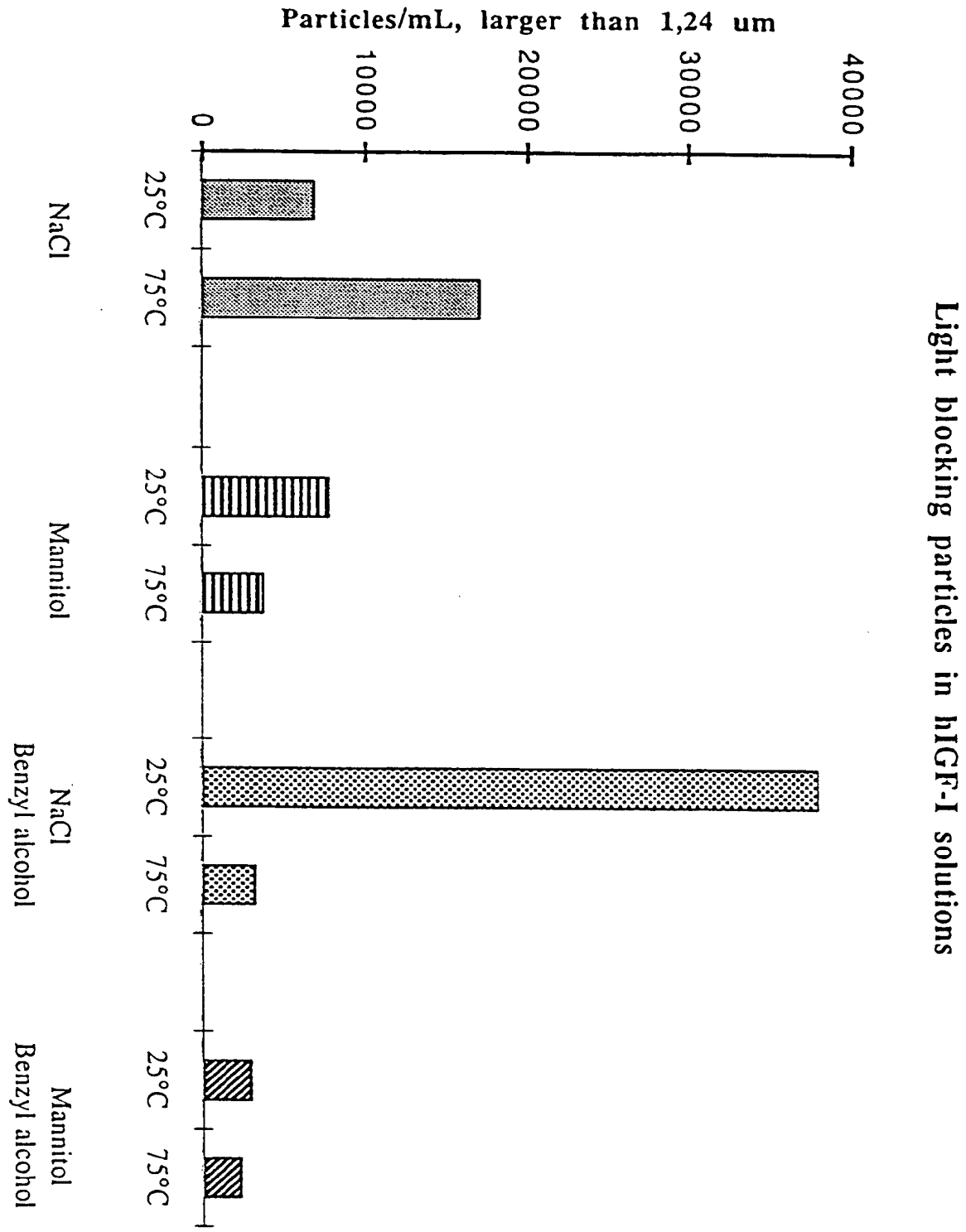


Figure 2

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 96/01041

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/30, A61K 47/26
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 8909614 A1 (GENENTECH, INC.), 19 October 1989 (19.10.89) -- | 1-6 |
| A | WO 9403198 A1 (GENENTECH, INC.), 17 February 1994 (17.02.94) -- | 1-6 |
| A | WO 9415584 A1 (PHARMACIA AB), 21 July 1994 (21.07.94) -- -------- | 1-6 |

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INTERNATIONAL SEARCH REPORT
Information on patent family members

28/10/96

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PCT/SE 96/01041

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
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| | | JP-T- | 8505617 | 18/06/96 |
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